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1. Your reference

MJWD/JM/B45229

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3. Full name, address and postcode of the or of each applicant (underline all surnames)

SmithKline Beecham Biologicals s.a.
Rue de l'Institut 89, B-1330 Rixensart, , Belgium

Patents ADP number (if you know it)

Belgian

6570956001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

Novel device

5. Name of your agent (if you have one)

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5800974004

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7605058001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and (if you know it) the or each application number

Country Priority application number Date of filing
(if you know it) (day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application Date of filing
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer yes if:

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 - b) there is an inventor who is named as an applicant, or
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Continuation sheets of this form

Description 19
Claim(s) —
Abstract —
Drawings 2

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Priority Documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 1/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11.

We request the grant of a patent on the basis of this application

Signature M J W Dalton Date 21-Jul-00

12. Name and daytime telephone number of person to contact in the United Kingdom

M J W Dalton 020 8975 6348

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Vaccines

The present invention relates to efficient devices for transdermal or intraepithelial administration of pharmaceutical agents to the human body, and in particular provides
5 devices for intradermal vaccination. The present invention provides skin patches having skin-piercing portion, and a suitable reservoir medium containing the agent to be delivered, wherein the reservoir is retained in or on the skin piercing portion. The patches are storage stable, and only substantially release the agent after penetration of the skin piercing portion into the skin. In a preferred embodiment, there is provided a
10 coated microneedle that releases the therapeutic agent directly into the skin after piercing the stratum corneum. In particular the agent is released during biodegradation of the reservoir medium in the underlying skin tissue. Further, vaccine patches are provided, methods of their manufacture, and their use in medicine.

15 The skin represents a significant barrier to external agents. A summary of human skin is provided in Dorland's Illustrated Medical Dictionary, 28th Edition. Starting from the external layers, working inwards, the skin comprises the epithelium comprising the stratum corneum, the viable epithelium, and underlying the epithelium is the dermis. The epithelium consists of five layers: Stratum corneum, Stratum lucidum, Stratum
20 granulosum, Stratum spinosum, and Stratum basale. The *epithelium* (including all five layers) is the outermost non-vascular layer of the skin, and varies between 0.07 and 0.12 mm thick (70-120 μ m). The epithelium is populated with keratinocytes, a cell that produces keratin and constitutes 95% of the dedicated epidermal cells. The other
5% of cells are melanocytes.

25 The stratum corneum dominates the skin permeability barrier and consists of a few dozen horny, keratinised epithelium layers. The narrow interstices between the dead or dying keratinocytes in this region are filled with crystalline lipid multilamellae. These efficiently seal the interstices between the skin or body interior and the
30 surroundings by providing a hydrophobic barrier to entry by hydrophylic molecules. The stratum corneum being in the range of 30-70 μ m thick.

Langerhans cells are found throughout the basal granular layer of the epithelium (stratum spinosum and stratum granulosum, (Small Animal Dermatology - Third Edition, Muller - Kirk - Scott, Ed: Saunders (1983)) and are considered to play an important role in the immune system's initial defence against invading organisms. This layer of the skin therefore represents a suitable target zone for certain types of vaccine.

Conventional modes for administration of pharmaceutical agents into or across the skin, most commonly by hypodermic needle and syringe, are associated with numerous disadvantages. Such disadvantages include pain, the requirement for trained professionals to administer the agent, and also the risk of needle-stick injuries to the administrator with the accompanying risk of infection with a blood born disease. As such, there is a need to improve the method of administration of all types of pharmaceutical into or through the skin.

In the past a number of alternative approaches have been described in order to overcome the problems of administering agent across the stratum corneum, including various designs of skin patches. Examples of skin patches which deliver agent through the skin without physically penetrating the stratum corneum layer include that described in WO 98/20734 and WO 99/43350. Other approaches where the skin is not punctured include electrotransport, or iontophoretic devices where the passage of agent is enhanced by the application of an electrical current into the skin. Many such devices are described in the literature (examples of which include US 6,083,190; US 6,057,374; US 5,995,869; US 5,622,530). Potential disadvantages of these types of non-penetration patches include the induction of significant sensitisation and discomfort during administration of the agent, and very poor uptake of antigen across the intact stratum corneum.

Other patches involving physical disruption or penetration of the skin have been described. Devices comprising liquid or solid reservoirs containing agent and a metal microblade patch have been described wherein the microblades physically cut through the stratum corneum to create pathways through which the agent can enter the

epithelium. Such devices are described in WO 97/48440, WO 97/48442, WO 98/28037, WO 99/29298, WO 99/29364, WO 99/29365, WO 00/05339, WO 00/05166, and WO 00/16833. Other devices involving puncturing of the skin include US 5,279,544, US 5,250,023 and US 3,964,482. Some of the disadvantages of these types of devices arise from generally poor rates of uptake of agent over the time of administration, despite the microblades penetrating the stratum corneum. The poor rates of uptake, results in long 'dwell times' during which the microblades are in contact with the skin. For conventional vaccination purposes, dwell times of longer than about fifteen to 30 minutes are relatively undesirable as they would prolong the period that the vaccinee needs to be monitored to check for possible side effects such as anaphylactic shock. In addition, many of the previously described products need to be transported and/or stored in refrigerated space. The larger volume of these products compared to vials means that fewer doses can be stored in the end-users refrigerators and making logistics more complicated and expensive.

15

The present invention provides for improved devices that are stable during storage, and are capable of administering and releasing agent efficiently into or through the skin. The invention is achieved by providing skin patches having a skin-piercing member having a plurality of protrusions that are loaded with a biodegradable reservoir medium containing the agent to be delivered, the loaded piercing protrusions, such as needles, being long enough and sharp enough to pierce the stratum corneum of the skin. Once the skin patch has been administered to the surface of the skin, and the coated protrusions or microneedles have pierced through the stratum corneum, the reservoir medium biodegrades thereby releasing the agent into the skin underlying the stratum corneum.

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The skin patches of the present invention may be used to administer any agent to a patient, which is desired to be administered in a short time frame in a painless manner without the dangers and fear often associated with conventional needles and devices. Examples of such agents include those agents that are required to be delivered daily, such as insulin, but also those agents that are required less frequently such as vaccines or genes for correction of genetic disorders. Vaccine coated patches form a preferred

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aspect of the present invention. In such applications the agent to be delivered is an antigen or antigens and may comprise micro-organisms or viruses (live, attenuated or killed) or gene or nucleic acid vectors (eg adenovirus, retrovirus), an antigen derived from a pathogen (such as a sub-unit, particle, virus like particle, protein, peptide, polysaccharide or nucleic acid) or may be a self antigen in the case of a cancer vaccine or other self antigen associated with a non-infectious, non-cancer chronic disorder such as allergy. The agent may be antigen alone or it may also comprise an adjuvant or other stimulant to improve and/or direct the immune response, and may also further comprise pharmaceutically acceptable excipient(s). The vaccine coated patches may be used for prophylactic or therapeutic vaccination and for priming and/or boosting the immune response. In cases of therapeutic vaccination where it is necessary to break tolerance then vaccine coated patches may be used as part of a specific regimen such as prime boost. Certain embodiments of the device described herein also have the significant advantage of being stored at room temperature thus reducing logistic costs and releasing valuable refrigerator space for other products.

The skin patches of the present invention can be used for a wide variety of agents that can not easily be administered using conventional patches such (as hydrophilic molecules) in the absence of penetration enhancers.

The skin piercing protrusions of the present invention may be made of almost any material which can be used to create a protrusion that is strong enough to pierce the stratum corneum and which is safe for the purpose, for example the protrusions may be made of a metal, such as pharmaceutical grade stainless steel, gold or titanium or other such metal used in prostheses, alloys of these or other metals; ceramics, semi-conductors, silicon, polymers, plastics, glasses or composites.

The patch generally comprise a backing plate from which depend a plurality of piercing protrusions such as microneedles or microblades. The piercing protrusions themselves may take many forms, and may be solid or hollow, and as such may be in the form of a solid needle or blade, or may be in the form of a microneedle having a hollow central bore. In this last embodiment, the central bore may extend through the

needle to form a channel communicating with both sides of the microneedle member (EP 0 796 128 B1).

The length of the piercing protrusion is typically between 1 μ m to 1mm, preferably
5 between 50 μ m and 600 μ m, and more preferably between 100 and 400 μ m. The length of the blade may be selected according to the site chosen for targeting delivery of the agent. The skin piercing patch member unit of the present invention may be take the form of, and be manufactured by the methods described in WO 97/48440, WO 97/48442, WO 98/28037, WO 99/29298, WO 99/29364, WO 99/29365, WO
10 00/05339, WO 00/05166, or WO 00/16833.

Where the piercing protrusion is solid, it may flat (termed microblade, see FIG 1) or may have a circular or polygonal cross section (see FIG 5). The protrusions can have straight or tapered shafts and may be flat or circular, or other polygonal shape, in cross
15 section. For example, the microblades may have a curved blade (FIG 3) or be formed into a V-section groove (FIG 6). Alternatively the protrusions may have more complex shapes to enhance adherence and fluid dynamics such as a five pointed star shown in FIG 7.

20 The piercing protrusions may be integral with the backing plate or may be attached thereto. In the case where the protrusions may be attached to the plate, the entire piercing protrusion may be formed of the reservoir medium. Such devices may be made by formed by drawing or extruding a molten reservoir medium containing the agent into fine points. For instance, molten reservoir medium could be cast directly
25 onto a backing plate through a multipore head, where the hot extrudate cools and sticks to the plate. When you draw back the extrudate a series of pointed ends is formed.

As a general feature of any piercing protrusion shape, in order to improve reservoir
30 adherence after coating, the surface of the protrusion may be textured. For example, the surface may be coarse grained, rippled or ribbed. In addition, solid microblades may further comprise holes (see FIG 4), such that the reservoir may dry therein and

create a reservoir tie, to hold the reservoir onto the blade more securely. In certain embodiments, including highly soluble and friable lyophilised formulations, it is preferred that the friable reservoir may be entirely held within such holes thereby protected from breakage during puncture of the skin.

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In an alternative embodiment the piercing protrusions may be separable from the base member. For example, in the embodiment where the piercing protrusions (or at least the tips thereof) is the reservoir itself, after penetration of the skin the piercing protrusions separates from the base support thus allowing the patch to be removed from the skin, whilst leaving the reservoir behind in the skin. The separation of the reservoir from the backing plate may be by physical shearing or by biodegradation of part of the needles adjacent the backing plate.

One embodiment of this may be to cast the microprotrusion tips out of a relatively poorly soluble disaccharide reservoir medium (containing a dispersion of the agent to be delivered) followed by casting the remaining portion of the microprotrusion and backing plate out of a relatively easily soluble material. Once inserted into the skin, the relatively easily soluble microprotrusion shaft would degrade away, thereby allowing the patch to be removed from the skin, whilst leaving the tips within the skin. The tips, remaining in the skin can then slowly release the agent by slower biodegradation.

The biodegradable agent reservoir may be any made from any medium that fulfils the function required for the present invention. The reservoir must be capable of adhering to the microprotrusion to a sufficient extent that the reservoir remains physically stable and attached during prolonged storage, and also remains substantially intact during the administration procedure when the coated microprotrusion pierce the stratum corneum. The reservoir must also be capable of holding or containing a suspension or solution of agent to be delivered in any dry or partially dry form, which is released into the skin during degradation of the reservoir medium.

Biodegradation of the medium in the sense of the present invention means that the reservoir medium changes state, such that changes from its non-releasing to its releasing states whereby the agent enters into the skin. The release of the active agent may involve one or more physical and/or chemical processes such as hydration, diffusion, phase transition, crystallisation, dissolution, enzymatic reaction and/or chemical reaction. Depending on the choice of reservoir medium, biodegradation can be induced by one or more of the following: water, body fluids, humidity, body temperature, enzymes, catalysts and/or reactants. The change of the reservoir medium may therefore be induced by hydration, and warming associated with the higher humidity and temperature of the skin. The reservoir medium may then degrade by dissolution and/or swelling and/or change phase (crystalline or amorphous), thereby disintegrating or merely increase the permeation of the medium.

Preferably the medium dissolves, and is metabolised or expelled or excreted from the body, but the reservoir may equally remain attached to the microprotrusion to be removed from the skin when the patch is removed. Examples of suitable reservoir media include, but are not restricted to, polyols such as sugars, polysaccharides, substituted polyols such as hydrophobically derivatised carbohydrates, amino acids, biodegradable polymers or co-polymers such as poly(hydroxy acid)s, polyahhydrides, poly(ortho)esters, polyurethanes, poly(butyric acid)s, poly(valeric acid)s, and poly(lactide-co-caprolactone)s, or polylactide co-glycolide. The coating of the microblades may be in the amorphous or crystalline state and may also be partially amorphous and partially crystalline. Particularly preferred reservoir media are those that stabilise the agent to be delivered over the period of storage. For example, antigen or agent dissolved or dispersed in a polyol glass or simply dried in a polyol are storage stable over prolonged periods of time (US 5,098,893, US 6,071,428; WO 98/16205; WO 96/05809; WO 96/03978; US 4,891,319; US 5,621,094; WO 96/33744). Such polyols form the preferred set of reservoir media. Preferred polyols include sugars, including mono, di, tri, or oligo saccharides and their corresponding sugar alcohols. Suitable sugars for use in the present invention are well known in the art and include, trehalose, sucrose, lactose, fructose, galactose, mannose, maltulose, iso-maltulose and lactulose, maltose, or dextrose and sugar alcohols of the aforementioned such as

mannitol, lactitol and maltitol. Other suitable excipients which may be included in the formulation include buffers, amino acids, phase change inhibitors ('crystal poisoners') which may be added to prevent phase change of the coating during processing or storage or inhibitors to prevent deleterious chemical reactions during processing or storage such Maillard reaction inhibitors like amino acids.

The reservoir medium is preferably of a solid or extremely viscous solution, which may itself be smooth or textured. For example, the medium may be solid, crystalline, amorphous/glassy, solid solution, solid suspension, porous, smooth, rough, or rugose.

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The formulations comprising the agent to be delivered and biodegradable reservoir medium are preferably mixed in aqueous solution and then dried onto the microprotrusion member or the formulation could be melted and then applied to the microprotrusion member. For example an aqueous solution of vaccine antigen and water soluble polyol may be prepared (such as trehalose), followed by coating the solution onto the microblades by dipping the member into the solution one or more times followed by drying at ambient temperature or lyophilisation to give a porous coating (repeating the process in part or whole to build up the depth of coating required, see FIG 2 – for a coated microblade (dotted area being reservoir medium – dashed lines showing that the reservoir medium may cover the entire undersurface of the microblade member)).

20

In an embodiment where the microneedles have hollow central bores (FIG 5A) or the microblades are curved or have a V-section (FIGs 3 and 6) once the blade is dipped into the liquid medium, the liquid solution will rise up and fill the bore or internal spaces by capillary action (for a microneedle having a central bore after loading with reservoir medium see FIG 5B).

25

Alternatively, minute picolitre volumes of solution or melted formulation may be sprayed onto individual blades by technology commonly used in the art of bubble-jet printers, followed by drying. An alternative method would be to prepare microspheres or microparticles or powders of amorphous formulation containing polyol such as

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sugar, using techniques known in the art (such as spray drying or spray freeze drying or drying and grinding) and by controlling the moisture content to achieve a relatively low glass transition temperature (for example 30°C), followed by spraying or dipping to bring the microspheres or microparticles or powders into contact with a
5 microprotrusion member heated to a temperature above that of the glass transition temperature of the microsphere (for example 45°C). The coated particles would then melt and adhere to the microprotrusion member and then dry or the coated microblade member would be further dried (to remove residual moisture content) thereby increasing the glass transition temperature of the reservoir medium suitable for
10 storage.

Alternatively, the microneedle member may be coated using a freeze coating technique. For example, the temperature of the microneedle member may be lowered below that of the freezing point of water (for example by dipping in liquid nitrogen)
15 and then aqueous solutions of the reservoir medium and agent may be sprayed onto the cold microneedles, or the microblade may be dipped into the solution of agent. In this way the agent and reservoir medium rapidly adheres to the microneedle member, which can then be sublimed by lyophilisation, or evaporated at higher temperatures, to dry the reservoir coating.

20 Another method to coat the microneedle members is to dip the microneedles in a solvent, such as water (optionally comprising a surfactant to ensure good contact) then dipping wetted blades in a powdered form of the reservoir medium which is soluble in the solvent, followed by drying to remove the solvent.

25 In the embodiment where the microblades comprise integral holes for dosing, strings of microblades (like a hacksaw blade) comprising individual blades like the one shown in figure 4, may be filled with reservoir and dried, before assembly into a patch. One such device assembled from many strings of blades is described in WO
30 99/29364. Alternatively, devices such as those described in WO 97/48440 may comprise integral holes, which may be filled whilst the blades are still in the plane of

the etched base plate, followed by the blades being punched into the perpendicular alignment with the reservoir medium in situ.

Preferably the vaccine formulations of the present invention contain an antigen or
 5 antigenic composition capable of eliciting an immune response against a human
 pathogen, which antigen or antigenic composition is derived from HIV-1, (such as tat,
 nef, gp120 or gp160), human herpes viruses, such as gD or derivatives thereof or
 Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus ((esp
 Human)(such as gB or derivatives thereof), Rotavirus (including live-attenuated
 10 viruses), Epstein Barr virus (such as gp350 or derivatives thereof), Varicella Zoster
 Virus (such as gpI, II and IE63), or from a hepatitis virus such as hepatitis B virus (for
 example Hepatitis B Surface antigen or a derivative thereof), hepatitis A virus,
 hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as
 paramyxoviruses: Respiratory Syncytial virus (such as F and G proteins or derivatives
 15 thereof), parainfluenza virus, measles virus, mumps virus, human papilloma viruses
 (for example HPV6, 11, 16, 18, ..), flaviviruses (e.g. Yellow Fever Virus, Dengue
 Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or Influenza virus
 (whole live or inactivated virus, split influenza virus, grown in eggs or MDCK cells,
 or Vero cells or whole flu virosomes (as described by R. Gluck, Vaccine, 1992, 10,
 20 915-920) or purified or recombinant proteins thereof, such as HA, NP, NA, or M
 proteins, or combinations thereof), or derived from bacterial pathogens such as
Neisseria spp, including *N. gonorrhea* and *N. meningitidis* (for example capsular
 polysaccharides and conjugates thereof, transferrin-binding proteins, lactoferrin
 binding proteins, PilC, adhesins); *S. pyogenes* (for example M proteins or fragments
 25 thereof, C5A protease, lipoteichoic acids), *S. agalactiae*, *S. mutans*; *H. ducreyi*;
Moraxella spp, including *M. catarrhalis*, also known as *Branhamella catarrhalis* (for
 example high and low molecular weight adhesins and invasins); *Bordetella spp*,
 including *B. pertussis* (for example pertactin, pertussis toxin or derivatives thereof,
 filamentous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis* and *B.*
 30 *bronchiseptica*; *Mycobacterium spp.*, including *M. tuberculosis* (for example ESAT6,
 Antigen 85A, -B or -C), *M. bovis*, *M. leprae*, *M. avium*, *M. paratuberculosis*, *M.*
smegmatis; *Legionella spp*, including *L. pneumophila*; *Escherichia spp*, including

- enterotoxigenic *E. coli* (for example colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof), enterohemorrhagic *E. coli*, enteropathogenic *E. coli* (for example shiga toxin-like toxin or derivatives thereof); *Vibrio spp.*, including *V. cholera* (for example cholera toxin or derivatives thereof);
- 5 *Shigella spp.*, including *S. sonnei*, *S. dysenteriae*, *S. flexnerii*; *Yersinia spp.*, including *Y. enterocolitica* (for example a Yop protein), *Y. pestis*, *Y. pseudotuberculosis*; *Campylobacter spp.*, including *C. jejuni* (for example toxins, adhesins and invasins) and *C. coli*; *Salmonella spp.*, including *S. typhi*, *S. paratyphi*, *S. choleraesuis*, *S. enteritidis*; *Listeria spp.*, including *L. monocytogenes*; *Helicobacter spp.*, including *H.*
- 10 *pylori* (for example urease, catalase, vacuolating toxin); *Pseudomonas spp.*, including *P. aeruginosa*; *Staphylococcus spp.*, including *S. aureus*, *S. epidermidis*; *Enterococcus spp.*, including *E. faecalis*, *E. faecium*; *Clostridium spp.*, including *C. tetani* (for example tetanus toxin and derivative thereof), *C. botulinum* (for example botulinum toxin and derivative thereof), *C. difficile* (for example clostridium toxins A
- 15 or B and derivatives thereof); *Bacillus spp.*, including *B. anthracis* (for example botulinum toxin and derivatives thereof); *Corynebacterium spp.*, including *C. diphtheriae* (for example diphtheria toxin and derivatives thereof); *Borrelia spp.*, including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (for example OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, OspC, DbpA, DbpB),
- 20 *B. andersonii* (for example OspA, OspC, DbpA, DbpB), *B. hermsii*; *Ehrlichia spp.*, including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis; *Rickettsia spp.*, including *R. rickettsii*; *Chlamydia spp.*, including *C. trachomatis* (for example MOMP, heparin-binding proteins), *C. pneumoniae* (for example MOMP, heparin-binding proteins), *C. psittaci*; *Leptospira spp.*, including *L. interrogans*;
- 25 *Treponema spp.*, including *T. pallidum* (for example the rare outer membrane proteins), *T. denticola*, *T. hyodysenteriae*; or derived from parasites such as *Plasmodium spp.*, including *P. falciparum*; *Toxoplasma spp.*, including *T. gondii* (for example SAG2, SAG3, Tg34); *Entamoeba spp.*, including *E. histolytica*; *Babesia spp.*, including *B. microti*; *Trypanosoma spp.*, including *T. cruzi*; *Giardia spp.*, including
- 30 *G. lamblia*; *Leshmania spp.*, including *L. major*; *Pneumocystis spp.*, including *P. carinii*; *Trichomonas spp.*, including *T. vaginalis*; *Schistosoma spp.*, including *S.*

mansoni, or derived from yeast such as *Candida spp.*, including *C. albicans*; *Cryptococcus spp.*, including *C. neoformans*.

Preferred bacterial vaccines comprise antigens derived from *Streptococcus spp.*,
 5 including *S. pneumoniae* (for example capsular polysaccharides and conjugates thereof, PsaA, PspA, streptolysin, choline-binding proteins) and the protein antigen Pneumolysin (Biochem Biophys Acta, 1989, 67, 1007; Rubins et al., Microbial Pathogenesis, 25, 337-342), and mutant detoxified derivatives thereof (WO 90/06951; WO 99/03884). Other preferred bacterial vaccines comprise antigens derived from
 10 *Haemophilus spp.*, including *H. influenzae type B* (for example PRP and conjugates thereof), *non typeable H. influenzae*, for example OMP26, high molecular weight adhesins, P5, P6, protein D and lipoprotein D, and fimbrin and fimbrin derived peptides (US 5,843,464) or multiple copy variants or fusion proteins thereof. Other preferred bacterial vaccines comprise antigens derived from *Moraxella Catarrhalis*
 15 (including outer membrane vesicles thereof, and OMP106 (WO97/41731)) and from *Neisseria meningitidis B* (including outer membrane vesicles thereof, and NspA (WO 96/29412)).

Derivatives of Hepatitis B Surface antigen are well known in the art and include, inter
 20 alia, those PreS1, PreS2 S antigens set forth described in European Patent applications EP-A-414 374; EP-A-0304 578, and EP 198-474. In one preferred aspect the vaccine formulation of the invention comprises the HIV-1 antigen, gp120, especially when expressed in CHO cells. In a further embodiment, the vaccine formulation of the invention comprises gD2t as hereinabove defined.

25

In a preferred embodiment of the present invention vaccines containing the claimed adjuvant comprise antigen derived from the Human Papilloma Virus (HPV) considered to be responsible for genital warts, (HPV 6 or HPV 11 and others), and the HPV viruses responsible for cervical cancer (HPV16, HPV18 and others).

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Particularly preferred forms of genital wart prophylactic, or therapeutic, vaccine comprise L1 particles or capsomers, and fusion proteins comprising one or more antigens selected from the HPV 6 and HPV 11 proteins E6, E7, L1, and L2.

- 5 The most preferred forms of fusion protein are: L2E7 as disclosed in WO 96/26277, and proteinD(1/3)-E7 disclosed in GB 9717953.5 (PCT/EP98/05285).

A preferred HPV cervical infection or cancer, prophylaxis or therapeutic vaccine, composition may comprise HPV 16 or 18 antigens. For example, L1 or L2 antigen
10 monomers, or L1 or L2 antigens presented together as a virus like particle (VLP) or the L1 alone protein presented alone in a VLP or capsomer structure. Such antigens, virus like particles and capsomer are per se known. See for example WO94/00152, WO94/20137, WO94/05792, and WO93/02184.

- 15 Additional early proteins may be included alone or as fusion proteins such as preferably E7, E2 or E5 for example; particularly preferred embodiments of this includes a VLP comprising L1E7 fusion proteins (WO 96/11272).

Particularly preferred HPV 16 antigens comprise the early proteins E6 or E7 in fusion
20 with a protein D carrier to form Protein D - E6 or E7 fusions from HPV 16, or combinations thereof; or combinations of E6 or E7 with L2 (WO 96/26277).

Alternatively the HPV 16 or 18 early proteins E6 and E7, may be presented in a single molecule, preferably a Protein D- E6/E7 fusion. Such vaccine may optionally contain
25 either or both E6 and E7 proteins from HPV 18, preferably in the form of a Protein D - E6 or Protein D - E7 fusion protein or Protein D E6/E7 fusion protein.

The vaccine of the present invention may additionally comprise antigens from other HPV strains, preferably from strains HPV 6, 11, 31, 33, or 45.

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Vaccines of the present invention further comprise antigens derived from parasites that cause Malaria. For example, preferred antigens from *Plasmodia falciparum*

include RTS,S and TRAP. RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P. falciparum* linked via four amino acids of the preS2 portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. Its full structure is disclosed in the International Patent Application No. PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S. TRAP antigens are described in the International Patent Application No. PCT/GB89/00895, published under WO 90/01496. A preferred embodiment of the present invention is a Malaria vaccine wherein the antigenic preparation comprises a combination of the RTS,S and TRAP antigens. Other plasmodia antigens that are likely candidates to be components of a multistage Malaria vaccine are *P. falciparum* MSP1, AMA1, MSP3, EBA, GLURP, RAP1, RAP2, Sequestrin, PfEMP1, Pf332, LSA1, LSA3, STARP, SALSA, PfEXP1, Pfs25, Pfs28, PFS27/25, Pfs16, Pfs48/45, Pfs230 and their analogues in Plasmodium spp.

The formulations may also contain an anti-tumour antigen and be useful for the immunotherapeutic treatment cancers. For example, the adjuvant formulation finds utility with tumour rejection antigens such as those for prostate, breast, colorectal, lung, pancreatic, renal or melanoma cancers. Exemplary antigens include MAGE 1 and MAGE 3 or other MAGE antigens for the treatment of melanoma, PRAME, BAGE or GAGE (Robbins and Kawakami, 1996, Current Opinions in Immunology 8, pps 628-636; Van den Eynde et al., International Journal of Clinical & Laboratory Research (submitted 1997); Correale et al. (1997), Journal of the National Cancer Institute 89, p293. Indeed these antigens are expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma. Other Tumor-Specific antigens are suitable for use with adjuvant of the present invention and include, but are not restricted to Prostate specific antigen (PSA) or Her-2/neu, KSA (GA733), MUC-1 and carcinoembryonic antigen (CEA). Accordingly in one aspect of the present invention there is provided a vaccine comprising an adjuvant composition according to the invention and a tumour rejection antigen.

Additionally said antigen may be a self peptide hormone such as whole length Gonadotrophin hormone releasing hormone (GnRH, WO 95/20600), a short 10 amino acid long peptide, in the treatment of many cancers, or in immunocastration.

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It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from *Borrelia sp.* For example, antigens may include nucleic acid, pathogen derived antigen or antigenic preparations, recombinantly produced protein or peptides, and chimeric fusion proteins. In particular the antigen is OspA. The OspA may be a full mature protein in a lipidated form virtue of the host cell (*E. Coli*) termed (Lipo-OspA) or a non-lipidated derivative. Such non-lipidated derivatives include the non-lipidated NS1-OspA fusion protein which has the first 81 N-terminal amino acids of the non-structural protein (NS1) of the influenza virus, and the complete OspA protein, and another, MDP-OspA is a non-lipidated form of OspA carrying 3 additional N-terminal amino acids.

Additionally the compositions of the present invention can include nucleic acids either in naked form or incorporated in a suitable vector such as adenovirus or retrovirus to aid incorporation of the nucleic acids into the cells of the skin after application. Applications of this embodiment include DNA vaccines and gene therapy products.

Vaccines of the present invention may be used for the prophylaxis or therapy of allergy. Such vaccines would comprise allergen specific (for example Der p1) and allergen non-specific antigens (for example peptides derived from human IgE, including but not restricted to the stanworth decapeptide (EP 0 477 231 B1)).

It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from a wide variety of sources. For example, antigens may include human, bacterial, or viral nucleic acid, pathogen derived antigen or antigenic preparations, tumour derived antigen or antigenic preparations, host-derived antigens, including GnRH and IgE peptides, recombinantly produced protein or peptides, and chimeric fusion proteins.

Vaccines of the present invention, may advantageously also include an adjuvant.

Suitable adjuvants for vaccines of the present invention comprise those adjuvants that are capable of enhancing the antibody responses against the IgE peptide immunogen.

- 5 Adjuvants are well known in the art (Vaccine Design – The Subunit and Adjuvant Approach, 1995, Pharmaceutical Biotechnology, Volume 6, Eds. Powell, M.F., and Newman, M.J., Plenum Press, New York and London, ISBN 0-306-44867-X).

Preferred adjuvants for use with immunogens of the present invention include aluminium or calcium salts (hydroxide or phosphate).

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Preferred adjuvants for use with immunogens of the present invention include: aluminium or calcium salts (hydroxide or phosphate), oil in water emulsions (WO 95/17210, EP 0 399 843), or particulate carriers such as liposomes (WO 96/33739).

- Immunologically active saponin fractions (e.g. Quil A) having adjuvant activity
 15 derived from the bark of the South American tree Quillaja Saponaria Molina are particularly preferred. Derivatives of Quil A, for example QS21 (an HPLC purified fraction derivative of Quil A), and the method of its production is disclosed in US Patent No. 5,057,540. Amongst QS21 (known as QA21) other fractions such as QA17 are also disclosed. 3 De-O-acylated monophosphoryl lipid A is a well known adjuvant
 20 manufactured by Ribi Immunochem, Montana. It can be prepared by the methods taught in GB 2122204B. A preferred form of 3 De-O-acylated monophosphoryl lipid A is in the form of an emulsion having a small particle size less than 0.2µm in diameter (EP 0 689 454 B1).

- 25 Adjuvants also include, but are not limited to, muramyl dipeptide and saponins such as Quil A, bacterial lipopolysaccharides such as 3D-MPL (3-O-deacylated monophosphoryl lipid A), or TDM. As a further exemplary alternative, the protein can be encapsulated within microparticles such as liposomes, or in non-particulate suspensions or aqueous solutions of polyoxyethylene ether of general formula (I)

- 30 $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{-A-R}$

wherein, n is 1-50, A is a bond or -C(O)-, R is C₁₋₅₀ alkyl or Phenyl C₁₋₅₀ alkyl (WO 99/52549).

Particularly preferred adjuvants are combinations of 3D-MPL and QS21 (EP 0 671 948 B1), oil in water emulsions comprising 3D-MPL and QS21 (WO 95/17210, PCT/EP98/05714), 3D-MPL formulated with other carriers (EP 0 689 454 B1), or QS21 formulated in cholesterol containing liposomes (WO 96/33739), or
5 immunostimulatory oligonucleotides (WO 96/02555).

Examples of suitable pharmaceutically acceptable excipients include water, phosphate buffered saline, isotonic buffer solutions.

Also adjuvant preparations comprising an admixture of either polyoxyethylene castor
10 oil or caprylic/capric acid glycerides, with polyoxyethylene sorbitan monoesters, and an antigen, are capable of inducing systemic immune responses after topical administration to a mucosal membrane (WO 9417827). This patent application discloses the combination of TWEEN20™ (polyoxyethylene sorbitan monoester) and Imwitor742™ (caprylic/capric acid glycerides), or a combination of TWEEN20™ and
15 polyoxyethylene castor oil is able to enhance the systemic immune response following intranasal immunisation. Novasomes (US 5,147,725) are paucilamellar vesicular structures comprising Polyoxyethylene ethers and cholesterol encapsulate the antigen and are capable of adjuvanting the immune response to antigens after systemic administration.

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Surfactants have also been formulated in such a way as to form non-ionic surfactant vesicles (commonly known as neosomes, WO 95/09651).

Other adjuvants which are known to enhance both mucosal and systemic
25 immunological responses include the bacterial enterotoxins derived from *Vibrio Cholerae* and *Escherichia Coli* (namely cholera toxin (CT), and heat-labile enterotoxin (LT) respectively). CT and LT are heterodimers consisting of a pentameric ring of β -subunits, cradling a toxic A subunit. Their structure and biological activity are disclosed in Clements and Finklestein, 1979, *Infection and Immunity*, 24:760-769;
30 Clements et al., 1980, *Infection and Immunity*, 24:91-97. Recently a non-toxic derivative of LT has been developed which lacks the proteolytic site required to enable the non-toxic form of LT to be "switched on" into its toxic form, once released

from the cell. This form of LT (termed mLT(R192G)) is rendered insusceptible to proteolytic cleavage by a substitution of the amino acid arginine with glycine at position 192, and has been shown to have a greatly reduced toxicity whilst retaining its potent adjuvant activity. mLT(R192G) is, therefore, termed a proteolytic site mutant. Methods for the manufacture of mLT(R192G) are disclosed in the patent application WO 96/06627. Other mutant forms of LT include the active site mutants such as mLT(A69G) which contain a substitution of an glycine for an alanine in position 69 of the LTA sequence. The use of mLT(R192G) as a mucosal vaccine is described in patent application WO 96/06627. Such adjuvants may be advantageously combined with the non-ionic surfactants of the present invention.

Other adjuvants or immunostimulants include the oligonucleotide adjuvant system containing an unmethylated CpG dinucleotide (as described in WO 96/02555). A particularly preferred immunostimulant is CpG immunostimulatory oligonucleotide, which formulations are potent in the induction and boosting of immune responses in larger animals. Preferred oligonucleotides have the following sequences: The sequences preferably contain all phosphorothioate modified internucleotide linkages.

OLIGO 1: TCC ATG ACG TTC CTG ACG TT (SEQ ID NO. 1)

OLIGO 2: TCT CCC AGC GTG CGC CAT (SEQ ID NO. 2)

OLIGO 3: ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG (SEQ ID NO. 3)

The CpG oligonucleotides utilised in the present invention may be synthesized by any method known in the art (eg EP 468520). Conveniently, such oligonucleotides may be synthesized utilising an automated synthesizer.

Alternatively polyoxyethylene ethers or esters may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, particles composed of polysaccharides or chemically modified polysaccharides, cholesterol-free liposomes and lipid-based particles, oil in water emulsions (WO 95/17210), particles composed of glycerol monoesters, etc.

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It is an intention of the present invention to administer agent or vaccine into the skin rapidly and with high yield of administration. This may be even further enhanced by a

number of means, comprising the use of highly soluble carbohydrates as the reservoir medium, and also by agitating and/or heating the microneedle member during administration.

- 5 The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 1-500 μg , more preferably 1-100 μg , of
- 10 which 1 to 50 μg is the most preferable range. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisations adequately spaced.
- 15 The formulations of the present invention may be used for both prophylactic and therapeutic purposes. Accordingly, the present invention provides for a method of treating a mammal susceptible to or suffering from an infectious disease or cancer, or allergy, or autoimmune disease. In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine. Vaccine preparation is
- 20 generally described in New Trends and Developments in Vaccines, edited by Voller, et al., University Park Press, Baltimore, Maryland, U.S.A. 1978.

- The formulations of the present invention may be used for both prophylactic and therapeutic purposes. In a further aspect of the present invention there is provided a
- 25 vaccine as herein described for use as a medicament.

FIG 1, Microneedle patch member

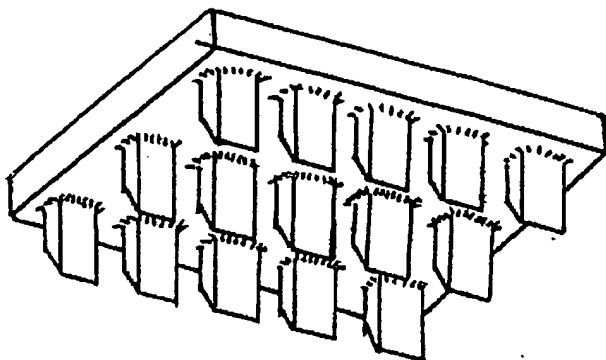


FIG 2, Cross section diagram of microneedle patch member of FIG 1, coated with a reservoir medium.

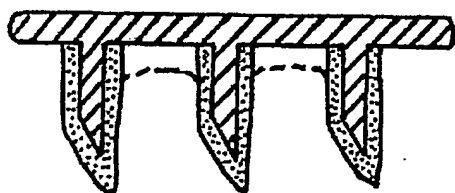


FIG 3,

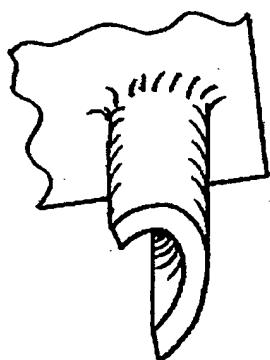
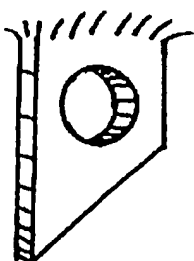


FIG 4

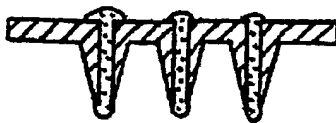


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FIG 5, A



B



5 FIG 6

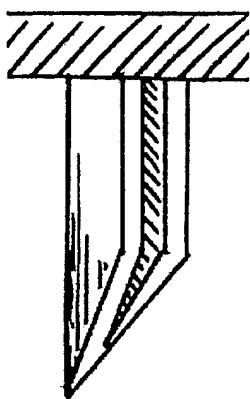
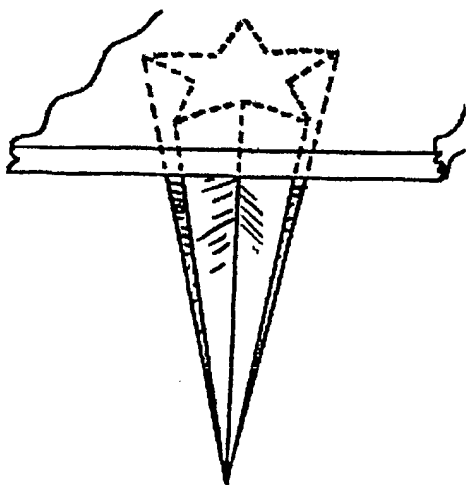


FIG 7,



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